54. The recombinant adenovirus of Claim 46 in which the region of the E4 early gene region which is deleted or mutated is open reading frame 6.

n.e.

Claim 55 canceled.

56. The recombinant adenoviral vector of Claim 46 or 47 which further comprises a deletion of the E3 gene region.

n.e.

57. The packaging cell line of Claim 48, 49, or 50 which supports the growth of the recombinant adenoviral vector which further comprises a deletion of the E3 gene region.

REMARKS

Attorneys for Applicant note with appreciation that the Examiner has indicated Claims 37-39 and 46-55 are in condition for allowance.

The claims have been amended as suggested by the Examiner to more particularly point out and distinctly claim the invention. Applicants also submit an executed Rule 132 Declaration by Dr. Wang. Applicants believe all the claims to be in condition for allowance.

In accordance with the duty of disclosure imposed by 37 C.F.R. §1.56 to inform the Patent and Trademark Office of all references coming to the attention of Applicants which are or may be related to patentability of the claimed invention, Attorneys for Applicant hereby direct the Examiner's attention to references AR to AX listed on the accompanying which recently came to the attention of the Attorneys of record.

Applicants do not believe the references AR to AX listed in the accompanying revised PTO 1449 form to be prior art to the above-identified application and therefore, not material to the patentability of the present application.

1. The Rejections Under 35 U.S.C. § 112. First Paragraph, Should be Withdrawn

The specification is objected to and Claims 46, 48, 49 and 54-55 are rejected under 35 U.S.C. § 112, first paragraph, for lack of enablement. The Examiner has maintained this rejection in the absence of an executed Declaration of Dr. Wang under 37 C.F.R. §1.132. Applicants submit herewith an executed Declaration by Dr. Wang and have reiterated below the arguments presented in the prior amendment dated November 12, 1997.

The Examiner asserts that the specification fails to enable the recited recombinant adenoviral vector deficient in E1 and E4 early gene regions in addition to the E2A early gene region and a packaging cell line which supports replication of a recombinant adenovirus deleted of the E1 early region gene, E4 early region gene, and in addition the E2A gene region. The Examiner's rejection is in error and should be withdrawn.

The specification describes adenoviral vectors which contain deletions of the E1 and E4 early region genes, in addition to the E2A gene region. The specification also describes novel adenoviral packaging cell lines to complement the E1, E4 and E2A gene regions deleted from the recombinant adenoviruses of the present invention. Plasmids containing the E4 and E2A gene regions under the control of inducible

promoters may be introduced into 293 cells, so that in an uninduced state expression of these cytotoxic genes is low enough to avoid toxicity to the host cell, but in an induced state is sufficiently activated to produce enough E4 and E2A to complement the replication-defective adenoviral vectors of the present invention. For example, see page 24, lines 5 to 27 of the instant application which describes the construction of a plasmid which contains the cytotoxic adenoviral E4 early gene region, deleted of its promoter, and placed under the control of an inducible mouse α inhibin promoter.

The Examiner's attention is invited to the accompanying executed Rule 132 declaration by Dr. Qing Wang (the "Wang declaration"), which describes the construction of a 293-derived packaging cell line which contains the cytotoxic gene regions, E4 and E2A, under the control of an inducible promoter, the mouse α-inhibin promoter. This cell line, designated 293-ORF6/E2A, has been constructed using the methods described in the instant application. The Wang declaration describes the construction of plasmids which provide the minimal essential region of E4 early region gene, open reading frame 6 (ORF 6) and the E2A gene region under control of an inducible promoter. The plasmids were introduced into a 293 cell line, which expresses the E1 early gene region. The successful introduction of the two cytotoxic gene regions into 293 cells, in addition to the E1 early gene region already present, is demonstrated by Southern blot analysis (see ¶6 of the Wang declaration). The ability of such a cell line to grow and survive is demonstrated by the observations that the positively identified cell lines may be

maintained for at least 12 tissue culture passage. The engineered cell line, 293-ORF6/E2A demonstrated similar growth characteristics to the 293 cell line engineered to express the E2A gene region, in addition to the E1 early gene region (see ¶7 of the Wang declaration). These results and observations demonstrate that a 293 cell expressing the E1 early gene region, in addition to E4-ORF6 and E2A under the control of an inducible promoter, may not only be successfully constructed, but may also be successfully grown and maintained in culture. The Wang declaration further demonstrates the 293-ORF6/E2A cell line may be constructed based on the disclosure of the above-identified application and that undue experimentation is not required by one skilled in the art to practice the invention as claimed. Therefore, the specifications under 35 U.S.C. §112 is fully enabling and the Examiner's rejection should be withdrawn.

2. The Rejections Under 35 U.S.C. § 112, Second Paragraph, Should be Withdrawn

Claims 39, 41-44, 48-50, 54 and 57 are rejected under 35 U.S.C.§ 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter of the invention. The claims have been amended to more particularly point out the invention as claimed, thereby obviating the Examiner's rejection. Thus, the Examiner's rejections under 35 U.S.C. § 112, should be withdrawn.

3. The Rejections Under 35 U.S.C. § 103 Should Be with Withdrawn

Claims 40, 41, 42, and 43 drawn to a DNA plasmid comprising an inducible promoter linked to nucleotide sequences encoding a cytotoxic gene product of adenovirus are rejected under 35 U.S.C. §103 as obvious over Ketner et al. in view of Jyan-Gwo et al. Claim 44 drawn to a DNA plasmid comprising a tetracycline responsive promoter linked to nucleotide sequences encoding a cytotoxic gene product of adenovirus is rejected under 35 U.S.C. §103 as obvious over Ketner in view of Jyan-Gwo and Gossen.

The Examiner contends that Ketner describes plasmids comprising a promoter linked to the adenoviral E4 early region, and that Jyan-Gwo and Gossen teach that α-inhibin and tetracycline responsive promoters are inducible promoters. The Examiner concludes that it would have been obvious to one of ordinary skill in the art at the time the invention was made to use the promoters described in Jyan-Gwo and Gossen and link them to the nucleotide sequences described in Ketner which encode the E4 early region, to arrive at the present invention. This rejection is in error for the reasons explained below.

A finding of obviousness requires a determination of the scope and content of the prior art, the level of ordinary skill in the art, the differences between the claimed subject matter and the prior art, and whether the differences are such that the subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere 383 U.S. 1 (1996). The proper inquiry is whether the art suggests the invention, and whether the art provides one of ordinary skill in the art with a reasonable

expectation of success. In re O'Farrell 853 F.2d 894, 7 USPQ2d 1673 (Fed. Cir. 1988). Both the suggestion and the reasonable expectation of success must be founded in the prior art and not in the Applicants' disclosure. In re Vaeck 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

In the present instance, the relevant inquiry is, first, whether the art relied on by the Examiner suggests the use of inducible promoters, such as cAMP-responsive promoters and tetracycline responsive promoters, to regulate expression of a cytotoxic adenoviral gene region. Moreover, assuming arguendo that the prior art provided such a suggestion, the second inquiry is whether it provides one of ordinary skill in the art with a reasonable expectation of success, i.e., that inducible promoters, such as the cAMP-responsive promoters or tetracycline-responsive promoters, are stringent enough to regulate expression of a cytotoxic adenoviral gene in a packaging cell, so as not to kill the cell. In re O'Farrell; In re Vaeck, supra. Applicants assert that the prior art neither suggests the plasmids of the present invention, nor gives any reasonable expectation of success.

The Examiner's rejection is based on several erroneous interpretations of the art cited. First, the Examiner contends that a person of ordinary skill in the art would recognize that the choice of an inducible promoter to drive expression of the E4 ORFs would simply be optimization of the process parameters of the system described in Ketner. There is no suggestion in Ketner to choose an inducible promoter to drive expression of the E4 ORFs. Ketner describes plasmids containing various E4 ORF deletions all of which are under the

control of an intact E4 promoter. Ketner uses these plasmids to transiently transfect 293 cells to achieve packaging of adenoviral E4 mutants. Ketner observes that low levels of packaging are achieved, and attributes this to "low efficiency of transfection, insufficient expression of E4 products from plasmids, low E4 gene dosage due to failure of the plasmid sequences to replicate with the viral genome, or a combination of those factors" (Ketner, page 3045, lines 20 to 22). Ketner recognizes that there is poor transfection efficiency and low levels of expression of E4, but Ketner does not suggest that the solution to this problem is an inducible promoter.

One of ordinary skill in the art may have recognized that in order to optimize the process parameters described in Ketner, one should improve transfection protocols or switch the E4 promoter for a stronger promoter in order to improve levels of E4 expression -- but why would one of ordinary skill in the art choose an inducible promoter to improve levels of E4 expression given the unimpressive levels of induction reported in the prior art for inducible promoters, (e.g., stimulation of the α-inhibin promoter only results in three fold induction in promoter activity (Jyan-Gwo). Thus, there is simply no motivation in the prior art to use an inducible promoter to improve the levels of E4 expression which is the goal desired by Ketner.

Further, one of ordinary skill in the art would not view an inducible promoter as an improvement of the process parameters of the system described in Ketner. Indeed, there is no motivation to combine Ketner with Jyan-Gwo and Gossen. Jyan-Gwo describes the characterization of a cAMP-responsive

promoter and Gossen describes a tetracycline-responsive promoter to regulate expression of a reporter gene, firefly luciferase. Ketner recognizes that there is a need to achieve higher levels of expression of E4 in a transient transfection assay -- not that there is a need to achieve tightly regulated expression of E4. Thus, one of ordinary skill in the art would select a stronger promoter, not an inducible promoter. Thus, there is simply no motivation to combine Jyan-Gwo and Gosser with Ketner.

Moreover, assuming arguendo that there was a suggestion in Ketner, or any other prior art reference, to use an inducible promoter to tightly regulate the expression of E4 due to its toxicity, there is no expectation of successfully using a cAMP-responsive promoter to achieve this goal, thus Claims 41 to 43 are separately patentable for additional reasons. The Examiner states that one would have been motivated to use the promoters of Jyan-Gwo since it was well known at the time that promoters containing cAMP-responsive elements inducibly regulate gene expression. However, there were many inducible promoters known at the time. Therefore, the suggestion lacking in Ketner is certainly not provided by Jyan-Gwo. Moreover, the prior art provides no expectation that cAMP-responsive promoters work sufficiently to control the toxicity of E4 gene products. One skilled in the art would choose an inducible promoter that when uninduced resulted in little or no expression of the toxic gene, so as not to kill the producer cell. In contrast to this type of inducible promoter, the invention describes cAMP-responsive promoters--i.e., promoters which are inducible by cAMP, but

which maintain a basal level of constitutive expression as reported by Jyan-Gwo, the secondary reference relied on by the Examiner (e.g., see Jyan-Gwo at pp. 298-299). Further, the cAMP-responsive promoter described in Jyan-Gwo only resulted in a three fold increase in reporter gene activity following induction, which is not a very impressive level of induction of promoter activity. Thus, one skilled in the art would not have expected a cAMP-responsive promoter to work sufficiently to control the toxicity of the E4 region gene products in a producer cell.

In view of the foregoing, the art relied on by the Examiner does not render obvious the plasmids of the claimed invention.

CONCLUSION

Applicants respectfully request entry and consideration of the foregoing amendments and remarks. Applicants believe the claims to be in condition for allowance.

Respectfully submitted,

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Laura A. Coruzzi (Reg. No.)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090

Enclosure